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A cluster-randomised controlled trial of the efficacy of indoor residual spraying with DDT against malaria in Gambian communities with high usage of long-lasting insecticidal mosquito nets

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Summary

Background Many malaria control programmes in sub-Saharan Africa use indoor residual spraying (IRS) with long-lasting insecticidal nets (LLIN), yet there is only weak evidence that this combination is better than LLIN alone. In a two-arm cluster randomized trial we assessed whether the combination provided a significantly different level of protection against clinical malaria in children or against house entry by vector mosquitoes.

Methods Clusters of Gambian villages were randomly allocated to LLIN alone (n=35) or LLIN and IRS with DDT (n=35). In each cluster 70-213 children, aged 6 months to 14 years, were surveyed at the start of the 2010 transmission season and followed in 2010 and 2011 by passive case detection for the primary endpoint of clinical malaria. This cohort was surveyed at the end of each transmission season to estimate the prevalence of *Plasmodium falciparum* infection and anaemia. Exposure to parasite transmission was assessed by collecting vector mosquitoes using both light and exit traps indoors. Data collection was blinded. In the IRS-LLIN arm, 85.1% houses were sprayed with DDT in year 1 and 81.6% in year 2. LLIN coverage in year 2 was 92.9% (3510/3777 children) in the IRS-LLIN arm and 95.5% (3622/3791) in the LLIN arm. In 2010, 7845 children were enrolled, 7829 completed the study, and 7533 (96.2%) had complete clinical and covariate data. In 2011, 7034 children remained in the study, 623 more were enrolled, 7657 completed the study and 7549 (98.6%) had complete data.

Findings Incidence rate of malaria over the two transmission seasons was 0.032 (95% CI 0.025-0.042) cases/child-month in clusters with LLIN and 0.031 (95% CI 0.026-0.043) with IRS-LLIN (P=0.59); the incidence rate ratio was 1.04 (95% CI 0.76-1.43), allowing for confounders and cluster size by regression analysis. Anaemia, parasite and spleen rates were similar in both study arms in both years. There was no significant difference in the density of vector mosquitoes caught in light traps in houses over the two transmission seasons; the mean *Anopheles gambiae* s.l per trap per night was 6.7 (95% CI 4.0-10.1) in the LLIN arm and 4.5 (95% CI 2.4–7.4, in the IRS-LLIN arm (P=0.128, random effects linear regression model).

Interpretation There was no significant difference in clinical malaria, anaemia, prevalence of infection or vector density between study arms. In this area with high LLIN coverage, moderate seasonal transmission and susceptible vectors, IRS did not provide additional benefit.

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Introduction

Over the past 10 years there have been unprecedented reductions in malaria in many parts of sub-Saharan Africa where there has been scaling-up of long-lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) [1]. The number of nets delivered in sub-Saharan Africa increased from 6 million in 2004 to 145 million in 2010, with 54% of households having at least one net in 2013 and about 36% of the population sleep under a LLIN [2]. Today universal coverage with either LLIN or IRS is the major malaria prevention strategy and in many settings where IRS is used, LLIN are already deployed. Whilst the individual protection afforded by LLIN [3] and IRS [4] is well known, the joint impact of these interventions is poorly understood [5, 6].

Theory suggests two possible outcomes from using this combination. Some models indicate that LLIN and IRS combined would interrupt transmission in areas of moderate transmission [7, 8]. Yet others suggest that the impacts could be antagonistic against the major African vectors: *Anopheles gambiae* s.s. [9] and *A arabiensis* [10]. The argument for an antagonistic effect centres on the mode of action of DDT used for IRS and the pyrethroids used for LLIN. DDT, the most persistent insecticide used for spraying [11], is considered both a spatial and contact repellent [12, 13]. If this is so, the repellent effect of DDT may reduce the contact of mosquitoes on LLIN and since LLIN reduce blood-feeding, fewer blood-fed mosquitoes may rest on sprayed surfaces.

Evidence on this critical question is contradictory. Results from experimental huts indicate that there is no additional benefit of using IRS if LLIN are in use [14] except where pyrethroid-resistant vectors occur, in which case a non-pyrethroid insecticide sprayed on the walls provides additional protection [15, 16]. However, a recent analysis of survey data from 17 African countries indicated these concerns might be unwarranted since using LLIN and IRS together was associated with lower malaria prevalence than LLIN alone [17] and a review of non-randomized studies indicated that addition of LLIN to IRS was associated with lower parasite rates than IRS alone [6]. Similarly a non-randomised field trial in Kenya found that using a combination of a pyrethroid IRS and LLIN provided 61% greater protection against the incidence of infection in children than LLIN alone [18].

Nonetheless, the only cluster-randomised controlled trial carried out to date showed that in Benin, when LLIN were targeted to pregnant women and under 6 year old children, there was no additional benefit from spraying homes with a carbamates insecticide against clinical malaria nor prevalence of infection [19]. Our study was designed to determine whether universal coverage with LLIN and DDT IRS combined provided better protection against clinical malaria than LLIN alone in a rigorous randomized controlled study.

Methods

Design

A detailed description of the study protocol has been published [20]. In brief, the main aim was to assess whether IRS with DDT and LLIN combined provide better protection against clinical malaria in children than LLIN alone. To address this aim, 70 clusters of Gambian villages , located over 2 km from neighbouring villages to avoid spill over, were randomly allocated to either LLIN alone or LLIN and IRS with DDT and children aged from 6 months to 14 years old were sampled according to cluster size and enrolled into a study cohort (Figure 1). These children were followed during the malaria transmission season in 2010 and 2011. Clinical malaria was recorded by study staff

using passive case detection (PCD) in close collaboration with government health workers both at the village and health facility levels. Parasites were detected by a rapid diagnostic test (RDT Paracheck Pf, Orchid Biomedical Systems, Goa, India) and treatment followed government treatment guidelines. The study cohort was surveyed for malaria indices (anaemia, parasite prevalence, and spleen rate) at the end of both transmission seasons to generate data for the secondary endpoints and also at baseline before the year 1 transmission season to assess possible imbalances in malaria at village level. Baseline data was used to compare the two groups before the interventions. Exposure to malaria vector mosquitoes and parasites indoors was assessed using standardized mosquito light and exit traps monthly from July to December in 16 village clusters in each study arm followed by identification of *A gambiae* and detection of sporozoite infection.

Study area and participants

The study was carried out in the Upper River Region, the far eastern region of The Gambia, and was based in the MRC Unit's field station in Basse (13.3167^{o} N, -14.2167^{o} W). This is a rural area of 1995 km^{2} of open Sudanian savannah with a single rainy season from May to October, followed by a long dry season. Malaria is highly seasonal with most malaria episodes experienced during or immediately following the rainy season; rainfall was above average in 2010 (1116 mm), and about average in 2011 (890 mm). This region is bisected by the river into the north and south banks. The population of the region was 182,586 in 2003, the most recent census. Almost half the residents enrolled into the study lived in houses with thatched roofs (49.31%, 1685/3417), the remainder were metal, and the most common inner wall surfaces was bare mud (50.48%, 1725/3417) and matt paint (41.41%, 1415/3417).

A total of 70 village clusters, consisting of one to three neighbouring villages, were enrolled with >110 children aged 6 months-14 years on 1st June 2010 and at least 2 km from a neighbouring village cluster to reduce the likelihood of spill-over of mosquitoes [21]. A study cohort was used to assess the impact of the intervention on malaria with a lower age range of 6 months, since infants would be partly protected by maternal immunity, and the upper limit was selected since many older children would have developed immunity to infection [22-25] and it was also the age at which many children move to schools further away from their village. These children were randomly selected using statistical software (STATA version 11.0), stratified by age (<5 years, 5-10 years and >10 years) and weighted towards the younger children, who were less immune, at a ratio of 2:2:1 to achieve a total of 7845 with an average of 111/cluster (range 65-213) (Figure 2). In June 2011, 318 children >14 years on 1st June 2011 were excluded from the cohort, and 490 children left the study (422 moved, 56 died and 12 withdrew consent). These were replaced by 636 children born in 2010 selected and stratified as in the first year of study. Informed consent was sought at the village level after sensitization meetings attended by village community leaders and health staff and all selected villages agreed to participate. Children were enrolled providing their carers/parents gave witnessed informed written consent and, for children who were able to understand at least some of the issues, providing they assented. Subjects and households were free to withdraw their participation at any time without giving a reason. If consent was not provided then replacement children were selected from a second enrolment list.

The impact of the intervention on the density of malaria vectors and their infection rate with malaria was monitored in 32 clusters, 16 in each study arm. In each cluster six rooms in six different compounds were selected

randomly, where CDC light traps and exit traps were placed one night each month from July to December both years.

Randomisation and masking

A total of 35 clusters (47 villages) were randomized to receive IRS with DDT, with 35 clusters (49 villages) receiving only LLIN. The protocol had stated that stratification would be by presence of a primary health care (PHC) in clusters but this criterion would not have given a balanced design since, although all settlements with PHC are large villages, the reverse is not true so cluster size was considered more logical. A computerised stratified randomisation scheme was used to balance cluster allocation based on cluster size, using the median and geographical area, by dividing the study area into four areas, two on each bank of the river. The 50 randomisations with the best balance from 100,000 randomisations were selected and numbered 1 to 50. A random draw of these was made by a member of the data safety monitoring board (DSMB) and the corresponding village allocation selected. Balanced randomization was used to enrol children of similar ages in each cluster with the target number enrolled increasing with village size (population <500: 75-115 enrolled, 500-1499:120-170, 1500 - 2258:180-190). Entomological sampling was conducted in a sub-set of clusters chosen from those nearer to the field station for logistical reasons and the households within each cluster were randomly selected.

Observer bias was reduced where feasible. Slide microscopists and their supervisors were blinded to the identity and intervention status of the subjects. Mosquito collector bias was reduced by using standardized traps, which do not rely on the ability of the fieldworker to collect specimens. Trap catches were examined by a different person to the trap collector and blinded to the trap location. Apart from data on IRS, no data forms or samples carried the group allocation and this was only added to the datasets after final cleaning.

Interventions

In clusters randomized to IRS, Hudson X-pert sprayers were used to apply DDT (DDT 75% WP, Hindustan Insecticides Ltd New Delhi, India) at a target dose of 2 g/m^2 to dwelling rooms from 15 to 28th July, 2010 and 20th July to 9th August, 2011 according to WHO guidelines (WHO 2007). The spray teams were experienced from national campaigns with operators from the Gambian National Malaria Control Programme and team leaders from the Upper River Regional Health Team. All team members received refresher training both years. Team leader duties included monitoring which rooms were sprayed, completing IRS data record forms and IRS cards for each house owner. The overall IRS supervisor (LBSJ) visited the spray teams daily to check coverage and discuss with the teams and the residents. All internal walls were sprayed, except those with gloss paint, and the inside surface of thatch roofs were sprayed. A random check of rooms sprayed was made by MRC field supervisors in year 2 by interviewing residents and inspection of record cards, with 47/49 households sprayed. Samples of DDT were analysed for compliance to WHO standards by an accredited laboratory and passed WHO/ SIF/1.R 9 specifications for appearance, DDT content, wettability wet sieving and suspensibility. During IRS, insecticidal sprays were sampled in 4-8 houses/Area on Whatman No. 4 filter papers under careful supervision to avoid over-spraying and

the insecticide concentration estimated using the high performance liquid chromatography Dionex Ultimate 3000 systems and software from Thermofisher Ltd Stafford House, Boundary Way, Hemel Hempstead, UK [26].

Concentrations were expressed as grams active ingredient/m² by reference to a standard curve. Persistence of insecticides on walls was measured using WHO cone tests (WHO, 2006) in six houses, stratified by wall surface at one, three and six weeks post-IRS using triplicate tests with an average of 21 DDT susceptible *A gambiae s.s.* M form per test [27]. LLIN were manufactured with permethrin at 2% w/w (Olyset Nets, Sumitomo Chemicals, Japan), in a factory which met WHO specifications, and residual activity of insecticide was determined in triplicate using WHO cone tests on six LLIN from a randomly selected cluster in each arm after they had been in use for 16 months.

Objectives and endpoints

The primary clinical objective was to assess whether IRS with DDT plus LLIN provided added protection against clinical malaria in children compared with LLIN alone. The primary entomological objective was to estimate the efficacy of IRS and LLIN combined on house entry by *A gambiae* s.l. compared with clusters with LLIN alone. The study was also designed to examine the efficacy of the double intervention in preventing anaemia and reducing malaria infection at the end of the transmission season each year. The clinical endpoints were haemoglobin concentration, frequency of moderate anaemia (defined as haemoglobin <80 g/L) and severe anaemia (haemoglobin <50 g/L), presence of malaria parasites, parasite density, frequency of high parasitaemia (≥5000 parasites/μL) and the prevalence of children with enlarged spleens. The primary endpoints were the incidence of clinical malaria assessed by PCD and number of *A gambiae* s.l. collected per light trap per night. Secondary endpoints were sporozoite rate estimations in trapped mosquitoes and estimated entomological inoculation rate (*i.e.* the mean number of infective mosquito bites per person per season).

Children in the cohort were monitored for residence in their villages for the duration of the PCD and if they were absent more than 50% of the time their data were censored from analysis. Less than 1% of the children met this criterion; in year1 36/3549 were censored in the LLIN arm and 24/3497 in the IRS-LLIN, and in year 2 46/3481 were censored in the LLIN arm, 36/3413 in the IRS-LLIN.

Clinical evaluations

Parents/carers of children enrolled in the cohort were encouraged to take their child to the nearest heath post or clinic if the child had fever. Twelve field assistants/nurses were posted to nine health clinics and three key health posts where they were responsible for working with government staff/village health workers to record cases of malaria in the cohort. Each staff member was responsible for five to eight clusters and their health posts, visiting the health posts at least once a week to collect data on cases and replenish supplies. Clinical malaria was defined as a child presenting at health facilities with an axillary temperature of ≥37.5 ^{o}C, or a history of fever in the past 48 h, together with the presence of *Plasmodium falciparum* parasites of any density detected by microscopy and/or RDT.

During the surveys children in the cohort were examined clinically for obvious symptoms and signs of illness, temperature and spleen enlargement. A sample of all the children in the cohort, at least 50/ cluster were randomly selected stratified by age, and these, as well as those reporting fever in the last 48 hours and/or with a temperature of

≥37.5 °C, were finger pricked for immediate measurement of anaemia, using a spectrophotometer (HaemoCue®), and presence of parasites by RDT. Only samples taken randomly were included in the analyses. Thick blood films were stained with Giemsa and examined under 1000-fold magnification by trained, experienced microscopists. Parasite counts were recorded per high power field and 100 fields counted before a slide was declared negative. Parasite density was estimated assuming that one parasite per high power field equals 500 parasites/µl [28]. Two slides were prepared from each subject, read separately by two experienced microscopists and discrepancies resolved by a third reader.

Entomological collections

Mosquito exposure was measured using standardized light and exit traps, which respectively estimate indoor-resting and exiting mosquitoes. Six sentinel rooms in 32 clusters, where a consenting adult slept under a bednet, were sampled monthly in both transmission seasons. Potential risk factors known to affect mosquito densities in The Gambia [29] were recorded at each collection. Mosquitoes were killed by freezing before morphological identification by standard keys [30, 31] and unfed and blood-fed mosquitoes were dissected to determine whether they were parous. All female *A gambiae* s.l. were processed for species determination using PCR [32] and *P falciparum* infection using an enzyme-linked immunosorbent assay [33]. Wild caught *A gambiae* s.l. larvae from Area 3 in 2010 and Area 1, 2, and 4 in 2011 were reared to adults and their susceptibility to permethrin and DDT was assessed using tube bioassays [34].

Sample size rationale

We hypothesised that LLIN would reduce incidence of clinical malaria by 50%, with LLIN and IRS combined reducing the residual incidence by 30-60% (i.e. 50% vs 65-80% fewer clinical cases of malaria). The study was designed to detect this difference at 80% power and 5% level of significance. Considering slide-positive parasite prevalence as a proportion and haemoglobin as a concentration, 35 clusters with 110 children each would have 80% power to detect a 30% reduction in parasitaemia and a 5 g/L increase in haemoglobin at the 5% level of significance if half the child cohort was sampled. Demonstrating a 60% reduction in house entering mosquitoes (*A gambiae*) associated with IRS-LLIN, with 90% power and at the 5% level of significance, required six houses in each cluster and 16 clusters in each arm of the trial over two years [35].

Statistical analysis

The final clean datasets were submitted to the statistician of the study Data Safety Monitoring Board on the 6th November 2012 before the data were unblinded and analysis followed the detailed analytical plan established on 30th March 2012. Clinical malaria was first examined by calculating incidence rates for each cluster, including multiple attacks in children if the second or third attack occurred at least 28 days after the onset of the previous attack; un-weighted mean ratios by year and study arm are presented. All subsequent analyses used incidence rates calculated over both malaria seasons and censoring at the first attack. These two-year incidence rates were calculated for each cluster and the mean rate ratio calculated by study arm with confidence intervals obtained using the approximations

given by Bennett [36]. Time to first malaria attack was examined by a survival analysis approach using Kaplan-Meier curves to compare the probability of subjects in the two arms becoming infected as the malaria transmission seasons progressed and significance was calculated using a log-rank test. Finally a random effects logistic regression with an offset for person-time was used to adjust for individual and cluster level covariate effects. Parasite rates and density and haemoglobin concentrations were estimated from community survey data averaging over clusters. Anaemia was defined using upper limits of 110 g/L for mild anaemia, 80 g/dL for moderate anaemia and 50 g/dL for severe anaemia, as stipulated in the Analytical Plan.

Differences in malaria transmission experienced in the two study arms were examined by comparing the mean number of vector mosquitoes caught indoors in sentinel rooms adjusted for clustering. Differences in number of mosquitoes caught in light-traps between study arms was estimated by multilevel analysis using a mixed effect model on square root transformed data with cluster as a random effect and the intervention and covariates as fixed effects. The sporozoite rate, with a 95% confidence interval, was estimated for each arm of the study and estimates of entomological inoculation rate (EIR) were calculated as the mean number of *A gambiae* s.l. /house/night multiplied by the sporozoite rate and the number of nights during the entomological survey period. The proportion of sporozoite infected mosquitoes was compared between the intervention arms using logistic regression. The effect of IRS with DDT on mosquitoes leaving sleeping rooms was quantified as the percentage of *A gambiae* s.l. caught in exit traps among the total mosquitoes caught in both traps using data from both seasons and the intervention arms were compared using Wilcoxon's rank test.

Ethical approval

The study was conducted in accordance with the principles set forth in the ICH Harmonised Tripartite Guideline for GCP and the Declaration of Helsinki in its current version, whichever affords the greater protection to the participants. It was approved by the Gambian Government/MRC Unit Joint Ethics Committee on the 12th August 2008 (ref: L2009.15, L2010.19; SCC1128) and the London School of Hygiene and Tropical Medicine Ethics Committee approved on the 16th September 2009 (ref: 5592). Trial Registration: ISRCTN01738840 - Spraying And Nets Towards malaria Elimination (SANTE). A Data Safety Monitoring Board reviewed the conduct and results of the trial. The only incentives given to households that participated in the trial were provision of LLIN and IRS, treatment of study children during the study and fares to reach referral clinics were refunded by study staff following known tariffs.

Role of the funding source

The sponsor of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

The population of 37,045 residents was evenly distributed between the two arms of the study (table 1) and across the four geographical study areas. The mean cluster sizes were also similar between the two arms (table 1), as was baseline bednet coverage (table 2), but ethnicity varied with more Mandinka and less Fula in the LLIN arm (table 1). These characteristics showed a similar distribution in the entomology clusters as in the entire study (table 1). House designs were similar in the two arms but with slightly more study children living in houses with thatched roofs in the IRS-LLIN arm than the LLIN arm.

There were 7845 children in the cohort in 2010 and 7657 in 2011 (figure 3). In 2010 outcome data were available for all children at baseline, 7829 (99.8%) during the PCD and 7105 (90.6%) at end of season, whilst in 2011 outcome data were available for 7657 (100%) during the PCD and 6895 (90.0%) at end of season (table 3). Enrolled children were evenly distributed by age and gender across the intervention arms (table 3). LLIN use was lower at baseline in the LLIN arm (50.9%) compared to the IRS-LLIN arm (58.5%), but parasite prevalence and density and anaemia prevalence were similar (table 3).

In clusters randomized to IRS, DDT was applied by three spray teams, each consisting of one supervisor and six or seven spraymen who sprayed an average of 220 rooms each day. IRS coverage per cluster was over 80% both years; both in the whole study and in the entomology clusters. To achieve this high coverage required repeated visits to clusters, in 2011 20% of clusters required more than 2 visits, Mean concentrations of DDT sprayed on the walls was close to the target dose of 2 g/m^2 (table 2). Residual activity of DDT, estimated by WHO cone tests in 2011, was high with 99.2% mortality (95% CI 97.2 to 100) week one post-IRS and 94.3% (95% CI 89.3 to 99.3) after six weeks. Estimations of DDT residual activity in a non-study village within the Upper River Region in 2011 using the same batch of DDT showed high levels five months post-IRS on both mud and matt painted walls, mean mortality of 92.5 and 94.7%, respectively [27].

LLIN were distributed under the auspices of the trial as recommended by the National Malaria Control Programme in The Gambia in 2010. During the household baseline survey in June 2010 householders reported that 60.3% of the sleeping places in their house had LLIN (table 2). In July 2010, LLIN were provided to those without a LLIN; 4527 were donated in the LLIN arm and 4696 in the IRS-LLIN. In August 2010, however, room to room surveys found only 49.0% sleeping places had LLIN in use, although 71.1% of the nets donated by the project were hung above sleeping places. In November 2010 a further 2138 and 1942 LLIN were provided to the two arms and in the March to April 2011 approximately 10,000 LLIN were provided by the national mass donation campaign. LLIN coverage in the child cohort at the end of year 1 was 3256/3543 (91.9%) in the LLIN arm and 3105/3492 (88.9%) in the IRS-LLIN arm (table 2). At the end of year 2, coverage rose slightly to 3622/3791 (95.5%) in the LLIN arm and 3510/3777 (92.9%) in the IRS-LLIN arm (table 2). The residual activity of permethrin estimated by WHO cone tests at 16 months post-donation was high with 91.0% mortality (95% CI 87.8 - 93.6) in a LLIN cluster and 89.3% (95% CI 85.8- 92.2) in an IRS-LLIN cluster; mortality on new LLIN was 92.8% (90.9 -94.5) and on an untreated net was 10.4% (7.4 - 14.1).

To examine the susceptibility of the local vectors to the study insecticides, in area 4 in 2010 and in areas 1, 2 and 3 in 2011, *A gambiae* s.l. larvae collected from breeding sites close to enrolled villages were raised to adults and

exposed to papers impregnated with permethrin, DDT or solvent at an average of 21.3 mosquitoes / tube. In both years, and from all sites, mortality in the controls tubes was always less than 4%. In 2010, in area 4, mortality to DDT and permethrin was 100% (97.5% CI 89.7-100), but few *A gambiae* s.l. were examined (table 4). In 2011, in areas 1-3, larger numbers of mosquitoes were examined and mortality rates to DDT and permethrin were less than 100% (mean range 88.3-94.8%, table 4).

The incidence of clinical malaria allowing for clustering and multiple attacks, was 0.047/child-month at risk (CMR) in the LLIN arm and 0.044/CMR in the IRS-LLIN arm in Year 1 and 0.032/CMR in the LLIN arm and 0.034CMR in the IRS-LLIN arm in Year 2 (table 5). Incidence of malaria over the two transmission seasons and censoring at the first clinical malaria attack, was 0.032 (95% CI 0.025-0.042) /CMR for the LLIN arm and 0.031 (95% CI 0.026-0.043)/CMR for the IRS-LLIN. The incidence rate ratio was 0.93, with 95% CI 0.65-1.42 using Bennett's approximation. Mixed effects logistic regression allowing for study area, eave status, net use and Fula ethnicity as fixed effects and cluster a random effect, gave an incidence rate ratio of 1.16 (95% CI 0.77-1.73). Mixed effects logistic regression allowing for study area, eave status, net use and Fula ethnicity as fixed effects and cluster as a random effect, gave an odds ratio of 1.04 (95% CI 0.76-1.43) .

Prevalence of parasite infection, measured at the end of both transmission seasons by surveys on children in the cohort, showed no difference between the study arms (table 5, t-test comparing *P falciparum* rates between study arms P=0.505, Year 1 and P=0.789, Year 2). Malaria infection prevalence increased with age and was higher in children residing in houses with open eaves and in those not using an LLIN (results not shown). Adjusting for these confounders, however, was without significant effect (logistic regression: odds ratio of *P falciparum* rates between arms of study Year 1, OR=1.27, 95% CIs 0.79-2.03 P=0.329, and Year 2, OR = 0.94, 95% CIs 0.60-1.47, P=0.789). Prevalence of moderate and severe anaemia was similar to the baseline values (tables 3 and 5) and there were no significant differences between the study arms (odds ratio of anaemia prevalence between arms of study by logistic regression, Year 1 OR=1.10, 95% CI 0.83-1.22, P=0.918; Year 2 OR 1.12, 95% CI 0.95-1.33, P=0.186).

All entomological collections were successful apart from one where the house was locked. All successful catches had covariate data. *A gambiae* s.l. were present in 36.4% of light traps (839/2303) and 9.0% of exit traps (207/2303). Over 94%, of mosquitoes were collected in light traps, the remainder from exit traps, and overall 37.1% (10,601/28,607) of those collected were anophelines of which 72.3% (7664/10601) were *A gambiae* s.l.; all the rest were culicines. Over 99% of the *A gambiae* s.l. were identified to species, 70.7% (5372/7596) were *A arabiensis* and the rest *A gambiae s.s.*

Densities of anophelines, *A gambiae* s.l. and the sibling species of this complex varied by year and were slightly lower in the intervention arm (table 6), but there was no significant difference between the intervention arms (linear regression allowing for clustering, Year 1, p=0.299; Year 2, p =0.341). The proportion of sporozoite positive *A gambiae* s.l. was low (table 6) with a significant interaction between study arms and year (logistic regression, P=0.039) but within year there was no significant difference between the arms (sporozoite rate difference Year 1= 0.0013, P=0.38; Year 2 = - 0.0056, P=0.06). There was also no significant difference in EIR between the two arms of the study (table 6). The influence of covariates on the primary entomological outcome (EIR) could not be examined due to the low sporozoite infection rates but their influence on mosquito catch size was possible. Linear

regression on numbers of *A gambiae* s.l. caught in light traps including year, the presence of open eaves, a tethered horse, matt painted walls and more than one sleeper in the room, showed no significant difference (P=0.281) between the study arms (adjusted mean caught over two years was 6.7, 95% CI 4.0 - 10.1, in the LLIN arm and 4.5, 95% CI 2.4 – 7.4, in the IRS-LLIN arm). IRS with DDT did not significantly influence the proportion of *A gambiae* s.l. leaving houses; the mean percentage that left in the LLIN arm was 11.7 and in the IRS-LLIN arm this was 8.8 (P=0.087 Wilcoxon rank test). In both arms of the study, the parity rate among *A gambiae* s.l. caught in light traps was high (table 6) and without statistical difference between the arms (linear regression allowing for clustering P=0.779).

Discussion

This study demonstrated that in an area of moderate seasonal transmission, with high coverage of LLIN, the addition of IRS did not reduce the level of clinical malaria experienced by study children. This conclusion is also supported by our entomological findings which show that the number of malaria vectors entering houses and the entomological inoculation rate was similar in both study arms. The incidence of clinical malaria, our primary clinical outcome measure, was similar in both study arms. The study arms were evenly balanced for cluster size, cluster distribution over the study area and coverage with the interventions. The enrolled child cohort was evenly balanced for age and gender, and also for net use, malaria infection and anaemia at baseline; drop-out rates were low and evenly balanced across the study arms. There was an imbalance in ethnicity, the IRS-LLIN arm had proportionally more Fula, an ethnic group previously associated with resistance to malaria [37]. Adjusting for ethnicity and other possible confounders in the multivariate model, however, gave no evidence that an effect of IRS was masked by confounders (unadjusted rate ratio of 0.93 compared to an adjusted rate ratio of 1.05). Importantly the secondary clinical endpoints of anaemia, *P. falciparum* infection rates, and prevalence of splenomegaly, were also similar between the two arms. Thus there was no evidence, from any of the additional malariometric parameters measured during the clinical studies, that the combination of IRS and LLIN together was different than LLIN alone in reducing malaria.

A subset of 32 clusters was sampled for the entomological endpoints as this was sufficient to detect a 60% reduction in house entering mosquitoes. The entomology clusters were also well balanced for cluster size and distribution over the study area and also had proportional more Fula. Over both years of the study, there were slightly fewer *A gambiae* s.l. entering houses in the IRS-LLIN arm, but these differences were not statistically significant either with unadjusted and adjusted analyses. This, together with similar entomological inoculation rates and the long-lived vector population, indicated by the high parity rates in both study arms, supports the clinical data and the conclusion that IRS with DDT offered no additional protection in the presence of high LLIN coverage

These results pose a question of major public health significance: why did the IRS intervention have no significant effect on malaria in this population where LLIN use was high? DDT is one of the most persistent insecticides used for spraying homes, being active for over six months (WHO 2006) and the residual activity found in this study and in a parallel study in the same area [27] documented effective activity at least up to five months, sufficient to cover the main transmission season in The Gambia. The spraying teams were experienced, well trained and supervised and achieved a high level of coverage of over 80% in both years. In addition, the measured

concentrations of DDT sprayed were within the expected range. One possibility is that mosquitoes in the study area were resistant to DDT. There is growing evidence that malaria vector control in sub-Saharan Africa is threatened by the spread of insecticide resistance in mosquitoes , both against the pyrethroids used for treating bednets [38] and all classes of insecticide used for IRS [39]. Twenty five years ago in The Gambia, shortly after permethrin-treated nets were introduced, little or no resistance to either DDT or permethrin was detected [40]. In 2008, two years before the present study, no resistance to either DDT (100.0% mortality, CI 82.4-100%) nor permethrin (100.0% mortality, CI 84.6-100%) was found in samples from around Basse town, located in the centre of the current study area, although only 19-22 mosquitoes were tested for each insecticide [41]. In 2010 we found similar results with adult *A gambiae* s.l. raised from larvae caught near study clusters east of Basse town (area 4). In 2011, larvae were caught in study areas 1-3, and the tube test results indicate low-level resistance to both insecticides used, with mean mortality of 88%, 89% and 91% to DDT and 93%, 93% and 94% to permethrin. In a pilot study which examined the possibility of using alternative insecticides to DDT for IRS, larvae were collected in two villages in study area 4 which were not enrolled in the present study. Here we found high levels of resistance to DDT and permethrin [27]. Overall these results indicate rising resistance but we conclude that over most of the study area resistance levels to DDT contact killing were low and were not the reason for a lack of effect of the intervention.

There are possible non-operational reasons for the lack of a significant effect. The effectiveness of DDT is thought to be partly due to its insecticidal activity, but it is also considered to be a spatial repellent, reducing the entry of mosquitoes indoors, and a contact irritant, increasing the rate at which mosquitoes leave a sprayed room [12]. Whilst we demonstrated high mortality of mosquitoes exposed directly onto DDT sprayed walls during WHO cones tests, there was no reduction in house entry, suggesting a lack of repellence. Our results also showed no difference in exit rates of *A gambiae* s.l. with and without DDT-IRS, suggesting a lack of contact irritancy from the sprayed walls. The coverage by LLIN was high in this study, over 83-95% coverage in children in the cohort; we note that the rates were lower in the survey where nets were directly observed. High coverage of LLIN may reduce the number of blood-feeding mosquitoes that would normally settle on the walls. Whatever the explanation for our finding, the result is that IRS did not contribute to increased protection.

There has been only one other published cluster-randomised trial examining the added benefit of combining IRS and LLIN [19]. The trial was conducted in Benin in 2008/9 and had four arms of seven villages each: in the baseline arm LLIN were provided to a targeted group, pregnant women and children under six years old (TLLIN), this was compared to universal coverage with LLIN (ULLIN), to ULLIN plus IRS with a carbamate insecticide, and to TLLIN plus carbamate sheeting. The main outcome measure was active case detection of malaria in a cohort of children conducted during 12 periods of six consecutive days at six weekly intervals. Clinical incidence varied over the four arms from 8.4 to 10.2 mean attacks per 100 children-months with no additional benefit of carbamate-IRS, or carbamate-sheeting, to LLIN. A non-RCT study in the western Kenyan Highlands also examined the additive benefit of IRS to high LLIN coverage and in addition examined the impact of targeted larviciding [42] by *post hoc* assignment of intervention and control to clusters. When LLIN coverage was high (92%), IRS with a pyrethroid insecticide had little additional benefit.

There were potential limitations to this trial design. Firstly, the communities could not be blinded to the interventions but subject bias would most likely lead to an under-reporting of clinical malaria in the study arm that received IRS, and thus would bias towards an increased effect of the intervention. Secondly, the village-clusters enrolled in the study were >2 km from neighbouring villages and in central Gambia it has been estimated that 90% of *A gambiae* s.l. bite within 1.6 km of their breeding sites [21] so although the current study design would have reduced spill-over, it could not totally avoid it. Thirdly, the village-clusters enrolled in the study were small (average population of 523, range 188 to 2645) with the dwelling houses close together surrounded by their agricultural fields. Mass-killing of mosquitoes would be more likely if the clusters were occupying a greater geographical area as this would further restrict the spill-over of mosquitoes from adjacent clusters or villages outside the study. However, the extremely high survival rates of mosquitoes in our study, parity rates of 77%, suggests that insecticide killing was low. Lastly, although our measurements indicate that resistance was not pronounced near study villages, one focus of high resistance to pyrethroid and DDT was recently detected close to the study area, and further studies are needed on the distribution of insecticide resistance.

Conclusion

In this study in an area of seasonal malaria transmission, with predominately susceptible vector populations, IRS did not provide any additional protection against malaria over high coverage of LLIN. The study indicates that IRS may not be beneficial in the increasing proportion of endemic areas where LLIN use is high.

Authors' contributions

SWL and MP conceived and designed the study. SWL was the trial director and MP was the trial coordinator/manager in The Gambia. MJ and LBSJ contributed to the design of the entomological collections and IRS application. LBSJ and BK advised on the interventions and the study communities; LBSJ lead the intervention teams. DJ provided the statistical input to the study. KB advised on clinical aspects of the trial, SK led these aspects. DC and UA contributed to the design and management of the study. MA and SC advised on and supervised the molecular analysis and microscopy, respectively. HK advised on carried out the HPLC analysis. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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***Figure* 1**: Schematic of study design.

***Figure* 2**: Flow chart of the child cohort

***Figure* 3**: Survival estimates over both transmission seasons

Table 1: Baseline characteristics of study clusters

Table 2: Baseline characteristics of the children enrolled at the beginning of the transmission in Year 1.

Table 3: Malaria outcome in the child cohort by treatment allocation

Table 4: Entomological characteristics by study arm:

Table 5: Insecticide susceptibility of *A gambiae* s.l. assessed by WHO tube tests

Table 1 Baseline characteristics of study clusters at the beginning of the transmission in Year 1.

Variable	LLIN only	IRS-LLIN
All study clusters (n=70)		
Total population:		
North Bank, west	5716	7272
North Bank, east	3791	2879
South Bank, west	4231	3763
South Bank, east	4686	4707
Total Population	18424	18621
Mean cluster population (± 95% CIs)	518 (407-629)	528 (389-668)
Ethnicity:		
Mandinka	60.30% (11109/18424)	42.53% (7920/18621)
Fula	28.91% (5326/18424)	43.49% (8098/18621)
Serrehule	7.52% (1385/18424)	8.47% (1578/18621)
Wollof & others	0.19% (36/18424)	0.11% (20/18621)
House features (presence of)		
Thatched roof	43.83% (3236/7383)	47.69% (3664/7683)
Mud walls	48.30% (3689/7637)	52.54% (3862/7350)
Matt-painted walls	39.81% (3040/7637)	38.82% (2853/7350)
Gloss-painted walls	1.96% (150/7367)	1.28% (94/7350)
Entomology clusters (n=32)		
Mean cluster population +/- 95%CI)	476 (345-608)	446 (356-535)
Ethnicity:		
Mandinka (%)	63.06% (4766/7558)	51.37% (3554/6919)

Fula (%)	30.43% (2300/7558)	46.83% (3240/6919)
Serrehule (%)	6.30% (476/7558)	1.81% (125/6919)
Wollof (%)	0.21% (16/7558)	0.0% (0/6919)

Eave status, open or closed, was recorded for children in the cohort in 2011. Open eaves were most common with 62.02% (2369/3820) in the LLIN arm and 59.13% (2269/3837) in the IRS-LLIN arm

Table 2. Interventions in the trial.

Variable	LLIN only	IRS-LLIN
Indoor Residual Spraying		
IRS coverage/cluster (95% CI) Year 1	-	86.50% (82.84-90.16%)
IRS coverage/cluster (95% CI) Year 2	-	82.77% (79.27-86.28%)
Mean DDT sprayed, g/m^2 (95% CIs) Year 1	-	1.69 (1.39-1.99)
Mean DDT sprayed, g/m^2 (95% CIs) Year 2	-	3.27 (2.39-3.96)
Long-lasting insecticidal nets		
Reported bednet* coverage, net/ sleeping place (%) June 2010, Year 1	61.86% (6698/10827)	58.81% (6289/10693)
Reported LLIN coverage in child cohort. Jan 2011	91.90% (3256/3543)	88.92% (3105/3492)
Reported LLIN coverage in child cohort. Jan 2012	95.54% (3622/3791)	92.93% (3510/3777)

* includes all net types

Table 3. Baseline characteristics of the enrolled children at the beginning of the transmission in Year 1.

Variable	LLIN only (n=3896)	IRS-LLIN (n=3949)
Female	49.51% (1929/3896)	48.57% (1918/3949)
Age in years, mean (95%CI)	6.11 (6.07-6.23)	6.18 (6.07-6.29)
Children using LLIN	50.90% (1983/3896)	58.52% (2311/3949)
Children using untreated bednets	13.96% (544/3896)	13.35% (527/3949)
Febrile children with positive RDT	1.68% (3/179)	0% (0/131)
Prevalence of mild anaemia (>80-110g/L)	34.24% (747/2179)	35.07% (735/2086)
Prevalence of moderate anaemia (>50-80 g/L)	3.61% (76/2179)	4.40% (91/2086)
Prevalence of severe anaemia (≤50g/L)	0.11% (2/2179)	0.14% (3/2086)
Haemoglobin g/L, mean (95%CI)	112.0 (111.3 –112.8)	112.5 (111.9 –113.2)
Pf parasite rate	1.61% (34/2163)	1.92% (35/2069)
Pf parasite rate (high parasitaemia, >5,000, parasites/μL,	0.00% (0/2163)	0.00% (0/2069)
Geometric mean parasite density per μL, mean (95%CI)	24.9 (12.2 - 50.9)	48.6 (29.6 -79.5)
Prevalence of enlarged spleens	4.58 (190/3892)	3.09 (114/3733)

Mean age of cohort in Year 2 (June 2011) was 6.39 years (6.27-6.50) in the LLIN arm and 6.39 (6.28 – 6.51) in the IRS-DDT. ; there were 47.89% (1830/3837) females in LLIN arm and 48.95% (1869/3818) in the IRS-LLIN.

Table 4. Insecticide susceptibility of *A gambiae* s.l. assessed by WHO tube tests

Year	Geographical area	Insecticide	*A gambiae* s.l.		% Mortality	95% CI
			Exposed	Died		
2010	4	DDT	34	34	100	89.72 - 100*
		Permethrin	35	35	100	90.00 - 100*
2011	1	DDT	118	105	88.98	81.90 -94.00
		Permethrin	75	70	93.33	85.12 - 97.80
	2	DDT	94	83	88.3	80.03 - 94.01
		Permethrin	46	43	93.48	82.10 - 98.63
	3	DDT	121	110	90.91	84.32 - 95.37
		Permethrin	58	55	94.83	85.62 - 98.20

* 97.25 % CI

Table 5. Malaria outcome in the child cohort by treatment allocation:
(arithmetic mean and 95% CI for continuous variables and number of children (%) for categorical variables unless otherwise specified.)

Variable	Year 1		Year 2	
	LLIN only (n = 3942)	IRS-LLIN (n = 3887)	LLIN only (n =3837)	IRS-LLIN (n =3820)
Passive case detection[a]				
Children in PCD with complete data	98.12% (3868/3942)	98.51% (3829/3887)	98.67% (3786/3837)	98.51% (3763/3820)
Children with 1 malaria attack	11.42% (450/3942)	10.52% (409/3887)	14.15% (543/3837)	13.61% (520/3820)
Children with >1 malaria attack	1.42% (33/3942)	0.85% (23/3887)	1.51% (58/3837)	1.31%[b] (50/3820)
Incidence of malaria /child-months at risk	0.0468 (0.0307-0.0630)	0.0442 (0.0312-0.0572)	0.0321 (0.0244-0.0398)	0.0341 (0.0243-0.0441)
Cross-sectional surveys[c]				
Pf parasite prevalence	14.92% (282/1979)	17.02% (334/1997)	17.35% (360/2083)	16.47% (345/2141)
Parasite prevalence >5,000 parasites/μL	0.95% (18/1979)	0.61% (12/1997)	1.24% (27/2083)	1.09% (22/2141)
Geometric mean parasite density per μL (s.d.)	34.56 (4.52)	46.14 (2.96)	62.46 (4.10)	67.46 (3.71)
Prevalence of mild anaemia (>80-110g/L)	41.36% (810/1981)	41.14% (825/2003)	42.79% (881/2068)	44.38% (940-2118)
Prevalence of moderate anaemia (>50-80 g/L)	5.42% (108/1981)	5.67% (114/2003)	4.33% (92/2068)	5.56% (115/2118)
Prevalence of severe anaemia (≤50 g/dL)	0.16% (3/1981)	0.22% (4/2003)	0.20% (4/2068)	0.21% (4/2118)
Mean haemoglobin g/L (95% CI)	112.7 (110.9-114.5)	112.5 (111.1-113.9)	111.3 (109.3-113.3)	110.9 (109.2-112.7)
Prevalence of enlarged spleen	3.09% (115/3534)	2.61% (83/3400)	0.36% (11/3409)	0.54% (19/3342)

a) Measurements in Year 1 were made only during the peak transmission season, whereas in Year 2 children were followed for the entire season.

b) In 2011, three children in the IRS-LLIN arm had three malaria attacks

c) Prevalence percentages are calculated using the means of the clusters and the overall totals are given in parenthesis beneath each

Table 6. Entomological characteristics by study arm:

Variable*	Light traps a)		Exit traps a)	
	LLIN only	IRS-LLIN	LLIN only	IRS-LLIN
Anophelines/trap/night Year 1	6.88 (3.90-9.89)[a]	4.80 (2.76-6.83)	0.62 (0.25-0.99)	0.47 (0.18-0.76)
Anophelines/trap/night Year 2	3.12 (1.22-5.02)	1.96 (0.26-3.67)	0.49 (0-1.18)	0.07 (0.02-0.12)
A gambiae s.l. /trap/night Year 1	4.92 (3.05-6.79)	3.70 (2.03-5.37)	0.54 (0.18-0.89)	0.40 (0.15-0.66)
A gambiae s.l. /trap/night Year 2	1.96 (0.69-3.24)	1.27 (0.39-2.15)	0.46 (0-1.15)	0.59 (0.01-0.10)
A gambiae s.s /trap/night Year 1	0.95 (0.58-1.33)	1.15 (0.65-1.65)	0.13 (0.06-0.21)	0.18 (0.02-0.34)
A gambiae s.s /trap/night, Year 2	0.66 (0.24-1.08)	0.60 (0.26-0.95)	0.16 (0-0.37)	0.02 (0-0.04)
A arabiensis /trap/night, Year 1	3.96 (2.31-5.60)	2.51 (0.21-3.81)	0.39 (0.09-0.69)	0.22 (0.09-0.35)
A arabiensis /trap/night, Year 2	1.27 (0.39-2.15)	0.67 (0.08-1.26)	0.28 (0-0.72)	0.03 (0.01-0.06)
Culicines/trap/night, Year 1	9.46 (4.55-14.37)	9.31 (0.47-18.16)	0.40 (0.14-0.66)	0.52 (0.01-1.04)
Culicines/trap/night, Year 2	6.46 (2.81-10.11)	4.63 (0.74-8.53)	0.21 (0.09-0.34)	0.11 (0.05-0.16)
Percentage of *A gambiae* s.l. with sporozoites, Year 1	0.32 (9/2829)	0.19 (4/2131)	N/A	N/A
Percentage of *A gambiae* s.l. with sporozoites, Year 2	0.09 (1/1131)	0.65 (5/773)	N/A	N/A
Entomological inoculation rate, Year 1	2.44 (0.69-6.39)	1.08 (0.16-4.02)	N/A	N/A
Estimated entomological inoculation rate, Year 2	0.29 (0.003-2.66)	1.45 (0.15-5.69)	N/A	N/A
Parity, Year 1	76.75% (72.41-81.08)	74.24% (68.56-79.92)	N/A	N/A
Parity, Year 2	79.86% (70.90-88.84)	83.42% (75.31-91.53)	N/A	N/A

*mean and 95% CI are presented for all data except for the percentage *A gambiae* s.l. with sporozoites, which are presented with the total infected mosquitoes / total assayed

a) Each arm and year there were 576 trapping collections, except in the IRS-LLIN in year 1 where there were 575.